Biochem. J. (1985) **228**, 653–660
Printed in Great Britain

Characterization of human tissue carnosinase

James F. LENNEY, Steven C. PEPPERS, Christine M. KUCERA-ORALLO and Robert P. GEORGE*
Department of Pharmacology, School of Medicine, University of Hawaii, Honolulu, HI 96822, U.S.A.

(*Received 13 December 1984/14 February 1985; accepted 21 February 1985*)

Human tissue carnosinase (EC 3.4.13.3) had optimum activity at pH 9.5 and was a cysteine peptidase, being activated by dithiothreitol and inhibited by *p*-hydroxymercuribenzoate. By optimizing assay conditions, the activity per g of tissue was increased 10-fold compared with values in the literature. The enzyme was present in every human tissue assayed and was entirely different from serum carnosinase. Highly purified tissue carnosinase had a broader specificity than hog kidney carnosinase. Although tissue carnosinase was very strongly inhibited by bestatin, it did not hydrolyse tripeptides, and thus appears to be a dipeptidase rather than an aminopeptidase. It had a relative molecular mass of 90000, an isoelectric point of 5.6, and a K_m value of 10mM-carnosine. Two forms of kidney and brain carnosinase were separated by high-resolution anion-exchange chromatography, although only one form was detected by various electrophoretic methods. Homocarnosinase and Mn^{2+}-independent carnosinase were not detected in human tissues, although these enzymes are present in rat and hog kidney.

Carnosinase (EC 3.4.13.3) was discovered by Hanson & Smith (1949), who found that pig kidney carnosinase was activated and stabilized by Mn^{2+} ions. Wood (1957) showed that carnosinase was widely distributed in the tissues of the rat. Rosenberg (1960*a*,*b*,*c*) purified hog kidney carnosinase and studied its activation and stabilization by metal ions. Zoch & Müller (1971) extracted carnosinase from human placenta, and Murphey *et al.* (1972) reported that human kidney, liver and spleen each contained two forms of carnosinase, which were separated electrophoretically. Lenney (1976) studied the specificity of hog kidney carnosinase and subsequently isolated a second carnosine-splitting enzyme (homocarnosinase) from hog kidney (Lenney *et al.*, 1977). Wolos *et al.* (1978) also reported the presence of two carnosine-hydrolysing enzymes in hog kidney. Margolis *et al.* (1979, 1983) isolated a Mn^{2+}-independent low-K_m carnosinase from mouse kidney, and studied the immunohistochemical localization of this enzyme in the tissues of the mouse. Lenney *et al.* (1982) characterized human serum carnosinase and showed that it was different from human tissue carnosinase: furthermore, they were unable to detect homocarnosinase in human tissues (Lenney *et al.*, 1983). A review of the literature shows that no systematic study of human tissue carnosinase has been published.

In the present paper, human tissue carnosinase has been isolated and characterized. This enzyme had an unusually high pH optimum and was strongly activated by dithiothreitol. It had a broader specificity than hog kidney carnosinase and appeared to be a true dipeptidase rather than an aminopeptidase.

Materials and methods

Materials

Peptides contained only the L-forms of amino acids, except for β-Ala-DL-Leu. Sephadex and a Mono Q HR 5/5 column were from Pharmacia Fine Chemicals (Piscataway, NJ, U.S.A.). A 200ml ultrafiltration cell and Diaflo YM30 membranes were from Amicon Corp. (Danvers, MA, U.S.A.). Ampholytes were from LKB (Gaithersburg, MD, U.S.A.), and homocarnosine and Gly-His-Lys were from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Other di- and tri-peptides, cytosolic and microsomal aminopeptidases and other reagents were from Sigma Chemical Co. (St.

* Present address: Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, U.S.A.

Louis, MO, U.S.A.). Bestatin, amastatin and arphaminine were gifts from Dr. H. Umezawa (Institute of Microbial Chemistry, Tokyo, Japan). Pancreatic dipeptidase was a gift from Dr. Y. Ito (Gifu College of Pharmacy, Gifu, Japan) and MK0791 was a gift from Dr. H. Kropp (Merck Institute, Rahway, NJ, U.S.A.).

Enzyme assays

To measure the activity of tissue carnosinase, the following solutions were measured into borosilicate (12mm × 75mm) digest and blank tubes by using positive-displacement pipettes: 0.20ml of 125mM-sarcosine/HCl buffer, pH9.5, 0.10ml of 10mM-dithiothreitol, and 0.10ml of an appropriately diluted enzyme solution containing 0.25mM-$MnCl_2$. (It is important to pipette the buffer first, otherwise enzyme inactivation may occur because of the acidity of dithiothreitol and $MnCl_2$.) After 15min for activation at 22°C, the reaction was started by adding 0.10ml of 100mM-carnosine (pH9.5) to the digest tubes. Digest and blank tubes were incubated at 30°C for 30min, and then 0.50ml of 0.6M-trichloroacetic acid was added to each tube and 0.10ml of substrate was added to the blank tubes. The quantity of histidine in the trichloroacetic acid supernatants was measured fluorimetrically, by its reaction with *o*-phthaldehyde as previously described (Lenney *et al.*, 1982).

The activity of Mn^{2+}-independent carnosinase was measured with 0.2mM-carnosine in 75mM-Tris/HCl buffer, pH8.0 (Margolis *et al.*, 1983). The histidine produced was measured by the *o*-phthaldehyde fluorimetric method.

In measuring the activity of carnosinase against a variety of di- and tri-peptides, a previously described ninhydrin procedure (Lenney *et al.*, 1977) was employed, in which the free amino acids were measured in the neutralized trichloroacetic acid supernatant.

Purification of tissue carnosinase

All procedures were conducted at 22°C. DEAE-cellulose (settled volume 215ml) was regenerated and equilibrated with 10mM-Tris/HCl, pH7.0, containing 0.1mM-$MnCl_2$ and 0.02% NaN_3. Frozen human kidney (40g) was homogenized in a blender with 210ml of this buffer. The suspension was centrifuged (10min at 8000*g*), and the supernatant was mixed with the DEAE-cellulose, with occasional stirring for 1h. This suspension was poured into a column (4cm diameter) and the bed was washed with the same buffer until the A_{280} fell to 0.05. Then a 450ml linear gradient of NaCl (0–0.5M) in this buffer was pumped through the column. The fractions containing carnosinase were pooled and concentrated to 32ml by ultrafiltration on a Diaflo YM30 membrane.

This concentrate was applied to a Sephadex G-200 column (4cm × 75cm bed) and was eluted with 10mM-Tris/HCl buffer, pH7.4, containing 0.1mM-$MnCl_2$ and 0.02% NaN_3. The fractions containing the enzyme were pooled and concentrated as above to 12ml. The resulting preparation contained 3.7mg of protein/ml, as measured by the method of Lowry *et al.* (1951).

Portions of this preparation were injected into a Mono Q HR 5/5 column equilibrated with 20mM-Tris/HCl, pH7.65, containing 10% (v/v) glycerol and 0.1mM-$MnCl_2$. The column, attached to a Beckman h.p.l.c. system, was eluted with increasing concentrations of NaCl at a flow rate of 1.0ml/min. To each fraction was added 0.1 vol. of 1mM-EDTA in 2mM-$MnCl_2$.

Distribution in various tissues

Tissue (1.0g) was ground with fine glass beads in a mortar with 5ml of 10mM-Tris/HCl buffer, pH7.5, containing 0.2mM-$MnCl_2$. The supernatant (after centrifugation for 10min at 15000*g*) was diluted 10-fold with this buffer, and 0.10ml was assayed for tissue carnosinase.

Electrophoresis and isoelectric focusing

Pevikon/Sephadex slab electrophoresis, polyacrylamide-gel electrophoresis and polyacrylamide-gel isoelectric focusing were conducted as previously described (Lenney *et al.*, 1982), except that carnosinase was assayed under the optimum conditions for the tissue enzyme. Isoelectric focusing was also conducted as described by Behnke *et al.* (1975). Tissue (2g) was homogenized with 3ml of water; the extract was dialysed overnight at 4°C against 0.1mM-$MnCl_2$, and 2ml was used in a 4ml sucrose gradient containing 2.5% ampholyte, pH3–10.

Results

Activation by dithiothreitol

Human tissue carnosinase appears to be a cysteine peptidase, because it was activated by dithiothreitol and inactivated by *p*-hydroxymercuribenzoate. The amount of activation varied from 2- to 6-fold, presumably depending on the degree of oxidation of the enzyme. Maximum activity was observed at approx. 2mM-dithiothreitol; this concentration was used in all routine assays.

pH–activity curve

In Fig. 1 the pH–activity curve of tissue carnosinase is compared with that of plasma (serum) carnosinase. Kidney, brain and liver carnosinases all displayed very similar pH–activity curves. In the tissue carnosinase assay at pH9.5,

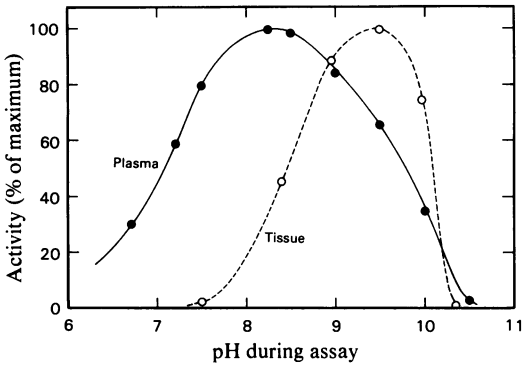

Fig. 1. *Effect of pH on the activity of tissue and plasma carnosinases*

The enzyme preparations were DEAE-cellulose column eluates; 30min assays were done with 20mM-carnosine in the presence of 0.05M-Tris/0.05M-sarcosine/HCl buffer mixtures, and pH values were measured in replicate digests.

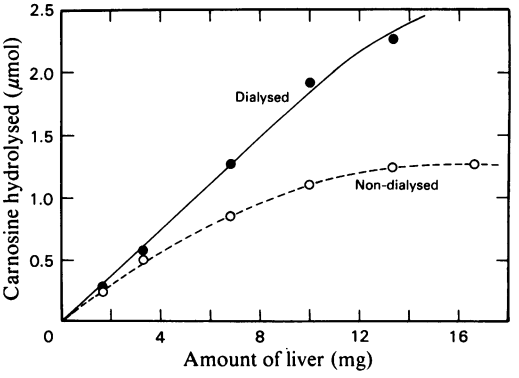

Fig. 2. *Effect of dialysis on the enzyme calibration curve of liver carnosinase*

A sample of liver was homogenized in 10mM-Tris/HCl buffer, pH7.5, containing 0.2mM-$MnCl_2$. A 10ml portion of this extract was dialysed overnight at 4° with stirring against 900ml of this buffer containing 5g of charcoal. Various amounts of the dialysed and non-dialysed extracts were then analysed for carnosinase activity.

sarcosine or 2-amino-2-methylpropane-1,3-diol buffers gave maximal activity, NH_3/HCl gave 20% less activity, and borate buffer was strongly inhibitory. A crude kidney extract and a purified brain preparation were analysed under many different conditions at pH7.2; the maximum activity observed was approx. 2% of the optimal activity at pH9.5.

Enzyme calibration curves

When various amounts of crude liver extract were employed in the assay for carnosinase, a non-linear enzyme calibration curve was obtained (Fig. 2). The shape of this curve suggests the presence of an endogenous inhibitor(s) (Dixon & Webb, 1979). After charcoal-facilitated dialysis of the extract, activity was increased and the calibration curve approached linearity, indicating almost complete removal of inhibitor(s). Partially purified kidney and brain preparations produced strictly linear enzyme calibration curves. In the routine assay of tissue for carnosinase activity, we found that the use of dilute non-dialysed extracts usually gave higher activity per g of tissue than the use of dialysed concentrated extracts. In certain tissues, carnosinase activity declined during dialysis, probably because of proteolysis.

Effect of metal ions on activity

Crude kidney and brain extracts were dialysed at 4°C against various metal salts (1mM) at pH7.0. The extracts dialysed against $MnCl_2$ were very active, whereas those dialysed against $CoSO_4$, $CdCl_2$, $CaCl_2$ or $ZnSO_4$ had little or no activity. In addition, $MnCl_2$ was much more effective than the other metal salts in reversing the inhibition of partially purified brain and kidney carnosinases by 0.2mM-EDTA. In the routine assay of tissue carnosinase, maximum activity was obtained with 50μM-$MnCl_2$ and 2.0mM-dithiothreitol. Higher concentrations of $MnCl_2$ were inhibitory.

Effect of metal ions and pH on stability

Partially purified brain carnosinase was heated at 50°C for 30min at pH7.5 in the presence of various metal ions (0.1mM). As compared with a control with no metal added, $MnCl_2$ increased thermostability, $FeSO_4$, $MgSO_4$ and $CaCl_2$ had no effect, whereas $CoSO_4$, $ZnSO_4$ and $CdCl_2$ decreased stability. As shown in Fig. 3, maximal stability at 50°C was observed at pH7.2–8.2. Thermostability varied slightly from one experiment to another, and no replicable difference between kidney and brain carnosinases was noted. Although Fig. 3 shows that stability at high pH values is poor, it was found that tissue carnosinase hydrolyses carnosine at a constant rate at pH9.5 during routine assays at 30°C, indicating that the enzyme is stable under assay conditions.

Effect of substrate concentration

Fig. 4 illustrates the effect of carnosine concentration on tissue carnosinase activity in the presence of 25μM-$MnCl_2$. When the data were analysed by using a s/v-against-s plot, brain and kidney carnosinases were found to have a K_m value of approx. 10mM. A K_m of 10mM was also obtained when the data in Fig. 4 were used in a computer program to generate the best-fitting hyperbola.

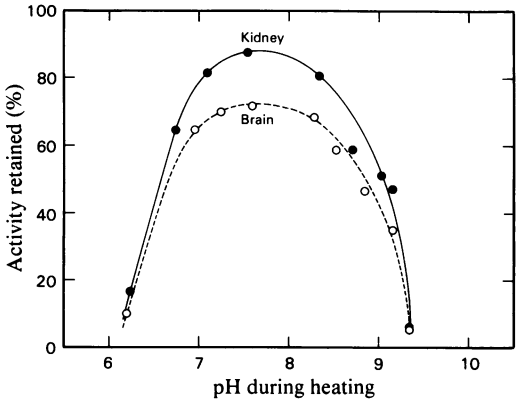

Fig. 3. *Effect of pH on the stability of tissue carnosinase at 50°C*

Portions of DEAE-cellulose column eluates were heated for 30min at 50°C in the presence of 0.8mM-$MnCl_2$ and a buffer mixture containing 5mM-acetate, 5mM-*N*-ethylmorpholine and 5mM-sarcosine. Residual activity was measured at pH9.5 by the routine assay procedure.

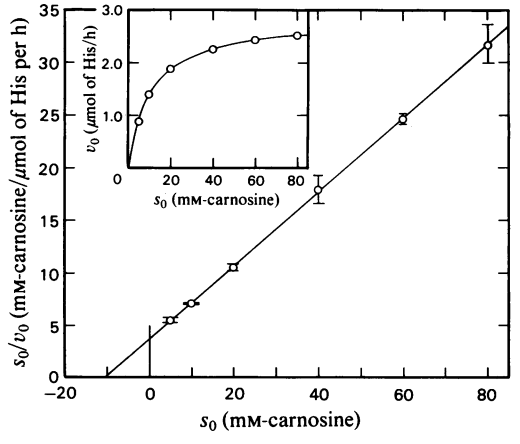

Fig. 4. *Effect of substrate concentration on the activity of brain carnosinase*

The data are plotted by a *s*/*v*-against-*s* plot and also as a Michaelis–Menten plot (inset). As carnosine concentrations were increased, more and more quenching of the histidine fluorescence occurred. The kinetic data were corrected for this effect by using histidine calibration curves prepared in the presence of various concentrations of carnosine.

However, when the $MnCl_2$ concentration was increased to 50μM, the K_m shifted to 23mM.

Relative molecular mass

When kidney or brain carnosinase was passed through a calibrated Sephadex G-200 column, each was found to have an apparent M_r of 90000.

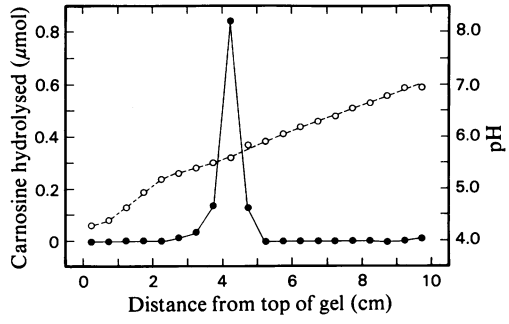

Fig. 5. *Isoelectric focusing of kidney carnosinase in a polyacrylamide-gel rod*

A DEAE-cellulose column eluate was employed in a 7.5%-acrylamide gel containing ampholytes of pH range 5–7. Focusing was at 410V for 3.5h at 8°C. Enzyme recoveries averaged 80%.

This result is in agreement with the value obtained for human kidney carnosinase by Murphey *et al.* (1972).

Electrophoresis in various media

Murphey *et al.* (1972) reported that human kidney, liver and spleen each contained two forms of carnosinase; two peaks of activity were observed when extracts of these tissues were subjected to starch-block electrophoresis at pH7.0.

When we subjected extracts of kidney and liver to electrophoresis under similar conditions in a Pevikon/Sephadex slab, only one peak of activity was detected. Crude extracts and partially purified preparations from kidney, brain and placenta were also subjected to non-denaturing polyacrylamide-disc-gel electrophoresis at pH8.9. In each case, one large tissue carnosinase peak ($m = 0.38$–0.48) was observed. In addition, a small peak ($m = 0.54$–0.62) representing serum carnosinase was detected (Lenney *et al.*, 1983). Small amounts of this enzyme were present because of the blood trapped in the tissue samples.

In addition, extracts of kidney, brain and liver were electrophoresed on vertical polyacrylamide- and starch-gel slabs at pH8.8. In each case, a single carnosinase spot was seen when the Lewis & Harris (1967) dipeptidase detection procedure was employed.

Isoelectric point

When tissue carnosinase was subjected to isoelectric focusing in a polyacrylamide gel, a single peak of activity at pH 5.6 ± 0.1 was observed (Fig. 5). Partially purified kidney and brain preparations showed a single sharp peak whether narrow- or broad-range ampholytes (pH3.5–10.0) were used. A similar result was obtained when

kidney or brain enzyme was focused in a sucrose gradient. A minor peak representing serum carnosinase was not observed because this enzyme is unstable at its isoelectric point (4.7). No peak of activity against homocarnosine was detected.

Inhibitors

Table 1 shows the effects of 12 inhibitors on the activity of tissue carnosinase. Bestatin, an inhibitor of certain aminopeptidases (Aoyagi *et al.*, 1977), was the most potent inhibitor, followed by amastatin. As shown in Fig. 6, bestatin contains leucine, and has a backbone chain identical with that of carnosine. Some other dipeptides containing leucine were also inhibitory. Phenylmethanesulphonyl fluoride, an inhibitor of serine proteinases, was inhibitory at 1mM; however, this compound inhibits papain by reacting with its essential thiol groups (Whitaker & Perez-Villasenor, 1968). Ala-His and Gly-Leu, substrates of carnosinase, inhibited the hydrolysis of carnosine.

The following compounds were not inhibitory; homocarnosine (0.4mM), anserine (0.4mM), arphaminine (10μM/ml), leupeptin (5μM), puromycin (4μM), MK0791, a dipeptidase inhibitor (10μg/ml), bacitracin (0.2mg/ml), spermine (0.8mM), spermidine (0.8mM), putrescine (0.8mM), cadaverine (0.8mM), sodium citrate (3.9mM), Na_2HPO_4 (10mM), adenosine (0.2mM), adenine (0.2mM), guanosine (0.2mM) and guanine (0.2mM).

Table 1. *Inhibition of tissue carnosinase*

Enzyme (DEAE-cellulose eluate) and inhibitor were combined and residual activity was assayed as described in the Materials and methods section. Inhibitor concentrations refer to those present during hydrolysis of the substrate.

Inhibitor	Concn.	Inhibition (%)
EDTA	0.4mM	100
p-Hydroxymercuribenzoate*	0.4mM	98
Phenylmethanesulphonyl fluoride	1mM	38
Bestatin	4nM	50
Amastatin	13μM	41
Leucine hydroxamate	80μM	93
Alanylhistidine	0.1mM	84
Alanyl-leucine	0.4mM	69
β-Alanyl-DL-leucine	0.4mM	44
Glycyl-leucine	0.4mM	71
Na_2CO_3	40mM	49
$CaSO_4$	3mM	35

* Dithiothreitol was omitted from experimental and control digests.

Purification

Human kidney carnosinase was purified by chromatography on columns of DEAE-cellulose, Sephadex G-200 and Mono Q, as described in the Materials and methods section. As shown in Fig. 7, two forms of tissue carnosinase were resolved on the Mono Q column. The enzyme was unstable after this step, but could be stabilized by adding $MnCl_2$ (to 0.3mM) and EDTA (to 0.1mM). The instability may have been caused by trace amounts of toxic metal ions picked up from the stainless-steel tubing of the h.p.l.c. apparatus.

In a similar Mono Q column experiment employing isocratic elution with 0.105M-NaCl, the first carnosinase peak was concentrated by vacuum dialysis and was subjected to polyacrylamide-gel electrophoresis at pH8.9. Coomassie Blue staining revealed three protein bands (*m* 0.23–0.28). In an unstained gel strip, carnosinase activity was detected in this region of the gel, but an exact correspondence of the enzyme to one of

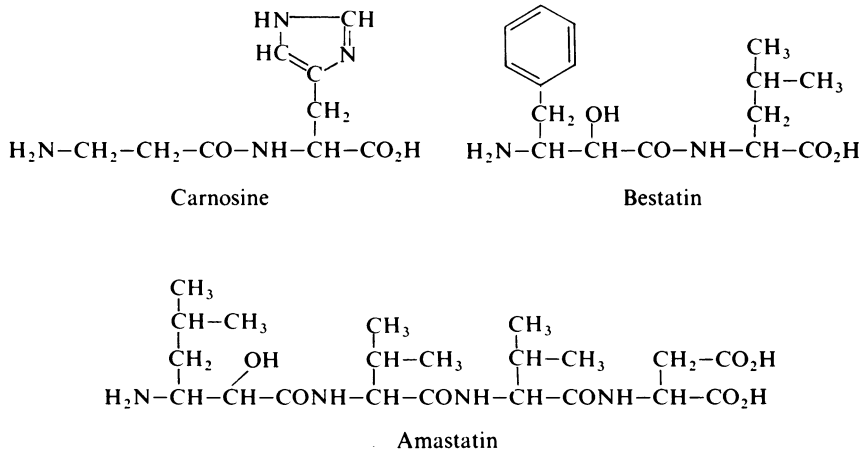

Fig. 6. *Structures of carnosine, bestatin and amastatin*

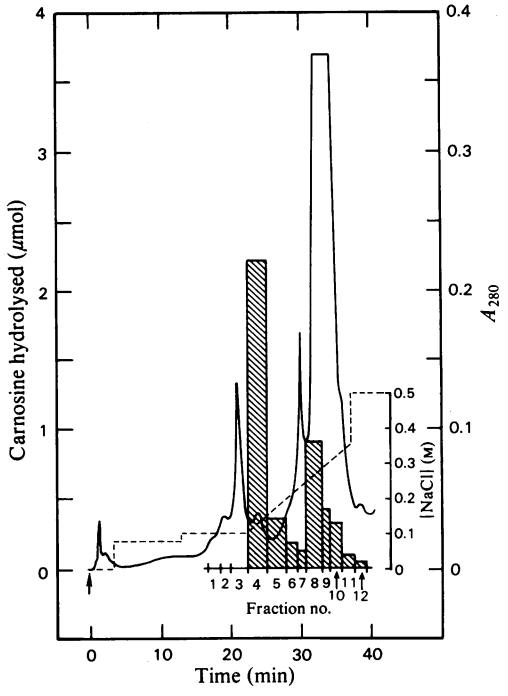

Fig. 7. *Mono Q column chromatography of human kidney carnosinase*

Enzyme (0.1 ml) was injected at zero time and eluted with increasing concentrations of NaCl (----). The shaded bars indicate the enzyme concentrations of the fractions, and the continuous curve represents the A_{280} of the eluate.

the protein bands was not established. The enzyme in the gel was very unstable and therefore could not be used for specificity studies.

Specificity

Another Mono Q column run by using isocratic elution was performed and each of the fractions in the first carnosinase peak was assayed to determine its activity against 13 different peptide substrates. The resulting profiles were not all of exactly the same shape, indicating the presence of contaminating exopeptidase activity. On the basis of the degree of parallelism of the curves, it was estimated that kidney carnosinase was more active against His-Ala, Gly-His and Gly-Leu than against carnosine, and less active against His-Gly and Ala-His than against carnosine. No activity was detected against homocarnosine, anserine, Gly-His-Gly, Gly-His-Lys, Gly-Gly-Gly, *N*-acetyl-His or benzyloxycarbonyl-Glu-Tyr.

By comparison, a sample of highly purified human pancreatic dipeptidase (Ito *et al.*, 1983) was active in hydrolysing Gly-His, Ala-His and Gly-Leu, but was inactive against carnosine. This

Table 2. *Distribution of carnosinase in human tissues*

Enzyme was extracted and assayed as described in the Materials and methods section. Results are means ± S.D. for the numbers of determinations in parentheses.

Tissue	Carnosinase activity (μmol/h per g)
Kidney	790 ± 220 (6)
Liver	300 ± 60 (4)
Spleen	220 ± 110 (6)
Cerebral cortex	105 ± 57 (4)
Lung	73 ± 31 (4)
Small intestine	49 ± 23 (3)
Placenta	49 (1)
Ovary	45 (1)
Testes	40 ± 19 (3)
Adrenal gland	27 (2)
Skeletal muscle	25 (2)
Heart	21 (2)
Pancreas	15 (2)
Erythrocytes (washed)	12 (1)
Pituitary	10 (1)
Uterus	9 (1)
Stomach	6 (1)

enzyme was inhibited by MK0791 (10 μg/ml), but was unaffected by bestatin (1 μg/ml). Cytosolic and microsomal pig kidney leucine aminopeptidases also failed to split carnosine.

Distribution of tissue carnosinase

Dilute extracts of 17 human tissues were analysed for carnosinase (Table 2), and activity was found in each. Kidney, liver, spleen and brain were the richest sources of this enzyme. Very little was found in the uterus, although this organ was the richest source of carnosinase in the rat (Lenney, 1976). The enzyme in washed erythrocytes was tissue carnosinase rather than the plasma isoenzyme, judging from its relative activities in the two assay methods. The concentrations of carnosinase in the kidney and liver were about 10 times higher than the values reported by Murphey *et al.* (1973).

Extracts of human kidney, brain, lung and spleen were analysed for Mn^{2+}-independent carnosinase. No activity was detected, although we found that this enzyme was readily measured in rat and hog kidney extracts.

Subcellular localization

Fresh rat kidney and liver and mouse liver were homogenized in 0.25 M-sucrose and fractionated centrifugally (Liao & Lenney, 1984). The Mn^{2+}-dependent carnosinase was found in the 100000*g* supernatant and not in the particulate fraction. Cathepsin B served as a marker for the particulate

fraction. Very fresh human tissues were not available, so frozen human placenta and kidney were fractionated by a similar procedure. Again, the carnosinase was found only in the cytosolic fraction.

Discussion

In Table 3 the properties of human tissue and plasma carnosinases are compared. The substantial differences between these enzymes show that they are isoenzymes (different gene products) rather than multiple forms of the same enzyme. Tissue carnosinase has a broader specificity than plasma carnosinase or hog kidney carnosinase (Lenney, 1976).

Tissue carnosinase was strongly inhibited by bestatin, which is an inhibitor of aminopeptidase B and leucine aminopeptidase (Aoyagi *et al.*, 1977). Nonetheless, tissue carnosinase appeared to be a dipeptidase, rather than an aminopeptidase. Although it was very active in hydrolysing Gly-His, it failed to split either of the peptide bonds in the closely related tripeptides Gly-His-Gly or Gly-His-Lys. Another dipeptidase inhibited by bestatin was an enzyme from *Streptococcus cremoris* (Hwang *et al.*, 1982).

In the literature it has tacitly been assumed that human serum and tissue carnosinases are similar to hog kidney carnosinase; assays were conducted under the conditions recommended for the pig enzyme by Hanson & Smith (1949) (pH8.0, Tris buffer, 37°C, Mn^{2+} as the activating metal ion). By reducing tissue carnosinase with dithiothreitol and analysing at pH9.5, activity was increased 5–15-fold. Crude extracts should be assayed by using dilute solutions, otherwise endogenous inhibitors will depress activity (Fig. 2).

Table 3. *Comparison of human carnosinases*

Property	Tissue carnosinase	Plasma carnosinase
Specificity	Broad	Narrow
Cysteine enzyme?	Yes	No
Optimum pH	9.5	8.5
pI	5.6	4.7
M_r	90000	160000
K_m		
Carnosine	10mM	4mM
Homocarnosine	–	0.4mM
Activated by	Mn^{2+}	Cd^{2+}
	Dithiothreitol	Citrate
Inhibited by	EDTA	EDTA
	p-Hydroxy-mercuribenzoate	Dithiothreitol
	Bestatin	Bestatin (weak)
	Borate	Tris

Tissue carnosinase has a small amount of activity at pH7.2; this increases sharply as the pH is raised to 9.5. Therefore we postulate that localized pH changes within the cytosol could serve to regulate the activity of this enzyme. Since our optimal assay conditions are non-physiological, the optimum pH for activity under conditions *in vivo* may be lower than 9.5. Hanson & Smith (1949) and Rosenberg (1960*b*) showed that activation of hog kidney carnosinase with Zn^{2+} in place of Mn^{2+} lowered the pH optimum markedly. Dithiothreitol, with its two thiol groups, is a metal chelator and probably helped to keep Mn^{2+} in solution at pH9.5 during the assay procedure; without chelators Mn^{2+} precipitates as the hydroxide at pH8.0 and above.

We have been unable to detect homocarnosinase in human tissues; our data indicate that plasma carnosinase is the only human enzyme capable of splitting homocarnosine (Lenney *et al.*, 1983) and anserine. Also we could not detect in human tissues the Mn^{2+}-independent low-K_m carnosinase found in certain strains of mice (Margolis & Grillo, 1984). Hog and rat tissues contain homocarnosinase and the Mn^{2+}-dependent and -independent carnosinases, but these animals do not have plasma carnosinase. Margolis *et al.* (1983) mentioned that the Mn^{2+}-dependent carnosinase of mouse tissues was partially membrane-associated and inhibited by bestatin, although no data were presented. Ganapathy & Leibach (1982) postulated the existence of a membrane-bound carnosinase in rabbit kidney. However, we were unable to detect particulate carnosinase activity in mouse, rat or human tissues.

Murphey *et al.* (1972) reported that human kidney, liver and spleen each contained two electrophoretic forms of carnosinase. One of these had a pI of approx. 7.0. We employed six different electrophoretic techniques and observed only one form of tissue carnosinase (pI5.6). The reason for this discrepancy is not known. However, two forms of this enzyme were separated by high-resolution anion-exchange chromatography (Fig. 7). The second form had a pI below 6.0 and there was preliminary evidence that it was derived from the first form by proteolysis. Both forms showed similar sensitivity to bestatin and other inhibitors. Our data indicated that human kidney, liver and brain carnosinases were identical.

We thank Richard W. N. Child and George R. Lenney for financial support, Anna M. Weiss, Paul W. H. Chan, Gary S. Rinzler and John L. Reardon for technical assistance, Dr. Charles Odom for human tissue samples obtained at autopsy, Dr. Y. Ito for a sample of pancreatic dipeptidase, and Dr. H. Umezawa for samples of bestatin, amastatin and arphaminine.

References

Aoyagi, T., Ishizuka, M., Takeuchi, T. & Umezawa, H. (1977) *Jpn. J. Antibiot.* **30S**, 121–131

Behnke, J. N., Dagher, S. M., Massey, T. H. & Deal, W. C. (1975) *Anal. Biochem.* **69**, 1–9

Dixon, M. & Webb, E. C. (1979) *Enzymes*, 3rd edn., pp. 53–54, Academic Press, New York

Ganapathy, V. & Leibach, F. H. (1982) *Biochim. Biophys. Acta* **691**, 362–366

Hanson, H. T. & Smith, E. L. (1949) *J. Biol. Chem.* **179**, 789–801

Hwang, I., Kaminogawa, S. & Yamauchi, K. (1982) *Agric. Biol. Chem.* **46**, 3049–3053

Ito, Y., Sugiura, M. & Sawaki, S. (1983) *J. Biochem. (Tokyo)* **94**, 871–877

Lenney, J. F. (1976) *Biochim. Biophys. Acta* **429**, 214–219

Lenney, J. F., Kan, S. C., Siu, K. & Sugiyama, G. H. (1977) *Arch. Biochem. Biophys.* **184**, 257–266

Lenney, J. F., George, R. P., Weiss, A. M., Kucera, C. M., Chan, P. W. H. & Rinzler, G. S. (1982) *Clin. Chim. Acta* **123**, 221–231

Lenney, J. F., Peppers, S. C., Kucera, C. M. & Sjaastad, O. (1983) *Clin. Chim. Acta* **132**, 157–165

Lewis, W. H. P. & Harris, H. (1967) *Nature (London)* **215**, 351–355

Liao, J. C. R. & Lenney, J. F. (1984) *Biochem. Biophys. Res. Commun.* **124**, 909–916

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Margolis, F. L. & Grillo, M. (1984) *Biochem. Genet.* **22**, 441–451

Margolis, F. L., Grillo, M., Brown, C. E., Williams, T. H., Pitcher, R. G. & Elgar, G. J. (1979) *Biochim. Biophys. Acta* **570**, 311–323

Margolis, F. L., Grillo, M., Grannot-Reisfeld, N. & Farbman, A. I. (1983) *Biochim. Biophys. Acta* **744**, 237–248

Murphey, W. H., Patchen, L. & Lindmark, D. G. (1972) *Clin. Chim. Acta* **42**, 309–314

Murphey, W. H., Lindmark, D. G., Patchen, L. I., Housler, M. E., Harrod, E. K. & Mosovich, L. (1973) *Pediatr. Res.* **7**, 601–606

Rosenberg, A. (1960*a*) *Arch. Biochem. Biophys.* **88**, 83–93

Rosenberg, A. (1960*b*) *Biochim. Biophys. Acta* **45**, 297–316

Rosenberg, A. (1960*c*) *Ark. Kemi* **17**, 25–40

Whitaker, J. R. & Perez-Villasenor, J. (1968) *Arch. Biochem. Biophys.* **124**, 70–78

Wolos, A., Piekarska, K., Glogowski, J. & Konieczka, I. (1978) *Int. J. Biochem.* **9**, 57–62

Wood, T. (1957) *Nature (London)* **180**, 39–40

Zoch, E. & Müller, H. (1971) *Enzymologia* **40**, 199–208